Name:____Justin Barry_____Date:____7-28-08_____ Period:_____ Lab Title:_____Elisa Lab___

Focus Question: Can you detect an antigen using enzyme-linked immunosorbent assay?

Word List:
-immunology
-antibodies
-antigens
-ELISA (enzyme-linked immunosorbent assay)
-secondary antibodies
-HRP, TMB
-controls
-false negative
-false positive

Hypothesis: Using an enzyme-linked immunosorbent assay, one can detect an antigen using a primary antibody, a secondary antibody, and a substrate.

Concept Map: (attached)

Overall Elisa Procedure:

1. Add sample and control sample to microplate strip.
2. Incubate for 5 minutes.
3. Add primary antibody to the wells and incubate.
4. Detect the bound antibodies with HRP-labeled secondary antibody.
5. Add enzyme substrate to the wells. Wait 5 minutes. Antigen present = blue; Antigen not present = colorless.

Procedures:

1. Label yellow tubes with initials and wells with appropriate initials.
2. Bind the antigen to the wells by transferring 50 microliters of + control into 3 wells. Repeat with – control into 3 different wells.
3. Transfer 50 microliters of each of your teams samples into initialed three wells.
4. Wait 5 minutes
5. Drain the microwells onto a paper towel.
6. Add wash buffer to each well. Discard paper towels
7. Repeat the addition of wash buffer.
8. Add 50 microliters of primary antibody into all 12 wells.
9. Wait 5 minutes
10. Wash primary antibody out of wells two times as before.
11. Add 50 microliters of secondary antibody into all 12 wells.
12. Wait 5 minutes
13. Wash secondary antibody out of wells three times as before.
14. Add 50 microliters of enzyme substrate into the 12 wells. Wait 5 minutes and record results.

Conclusion: You can detect an antigen using an enzyme-linked immunosorbent assay by allowing the antigen to bind to the plastic well via hydrophobic interactions, by the addition of a primary antibody, by the addition of a secondary antibody, and by the addition of an enzyme substrate. The positive test will reveal a blue solution.

Data and Analysis:
Part I: My protein sample contained a blue solution (positive test for the antigen).
Part II: My protein sample contained a blue solution (positive test for the antigen).
Part III: My protein sample was colorless, indicating that there was no presence of the antigen.

Questions:
See attached sheet

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Questions Part I:
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11. The proteins will bind via hydrophobic interactions to the well if the antigen was present. If the antigen was not present, the proteins did not bind to the wall.
12. You needed to wash the wells to get rid of any extra protein or antibody that did not bind to the wall or bind to the previously added mixture.
13. If my sample contained the antigen the PA would bind to the antigen in the wells. If the antigen was absent, the PA would nto bind to the antigen/wells.
14. If my sample contained the antigen, the SA would bind to the PA. If no antigen were present the SA would not bind to the PA.
15. Not necessarily. The assay might not have worked properly. There also could have been human error involved. The reagents could have also degraded.
16. You assay samples in triplicate to eliminate the possibility of human error.
17. Home pregnancy, ovulation, and drug tests use antibody-based tests. You can buy all three of these at your local pharmacy.
18. Not necessarily. You could have been in contact with a carrier of the disease. The transmissibility of a disease can vary from disease to disease. In a population, one can obtain a disease from the original infected person or a carrier.

Through deductive reasoning <u>Jen (#4) and Joe (#9)</u> were the two individuals who started the infection in part I of the lab.

Questions Part II:
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1. My sample did contain the antigen because the solution turned blue.

Questions 2-8: see questions 11-18 above for the repetitive answers to the questions.

Questions Part III:
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9. My serum did not have the antibodies because all the solutions with my initials were colorless at the end of the experiment.
10. If you tested positive for antibodies, you may not necessarily have been exposed to the disease. The result could have been a false positive. Since my result was dark positive, it is most likely that I was positive for antibodies.
11. If I had a positive test but did not have the disease there could have been contamination with the antigen. Human error also could have been the problem.
12. Repeat from part I.
13. If the sample was positive, the serum antibodies attach to the antigen. If the sample was negative the serum antibodies did not attach to the antigen.
14. Repeat from part I.
15. When you added the SA, the serum sample was positive if the SA attached to the PA. If a sample is negative, the SA did not attach to the PA.
16. Repeat from part I.